

Epidemiology of *Escherichia coli* O157 in Feedlot Cattle

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ABSTRACT

Fecal samples from cattle in 100 feedlots in 13 states were bacteriologically cultured for *Escherichia coli* O157 that did not ferment sorbitol, lacked beta-glucuronidase, and possessed genes coding for Shiga-like toxin. In each feedlot 30 fresh fecal-pat samples were collected from each of four pens: with the cattle shortest on feed, with cattle longest on feed, and with cattle in two randomly selected pens. *E. coli* O157 was isolated from 210 (1.8%) of 11,881 fecal samples. One or more samples were positive for *E. coli* O157 in 63 of the 100 feedlots tested. *E. coli* O157 was found at roughly equal prevalence in all the geographical regions sampled. The prevalence of *E. coli* O157 in the pens with cattle shortest on feed was approximately threefold higher than for randomly selected and longest on feed pens. Of the *E. coli* O157 isolates found in this study, 89.52% expressed the H7 flagellar antigen. *E. coli* O157 was found to be widely distributed among feedlot cattle, but at a low prevalence, in the United States.

Key words: *E. coli* O157, feedlot, cattle, epidemiology

Serious human illnesses associated with *Escherichia coli* O157:H7, including bloody diarrhea and hemolytic uremic syndrome, have been reported with increasing frequency since this organism was first reported in 1982 (2, 6, 8, 10, 15, 16). Cattle have been implicated as a reservoir of this agent by direct culture of the organism from foods of bovine origin, prevalence studies in cattle, and by epidemiologic associations with outbreaks of *E. coli* O157:H7-associated disease in humans (1, 3, 4, 11, 17). Most epidemiological studies on *E. coli* O157 in cattle have focused on dairy operations, but limited sampling has also been done in beef cattle (3). Studies on dairy cattle have determined that there are differences in prevalence of cattle excreting *E. coli* O157 among herds and associations between prevalence in cattle and herd management practices (1, 3, 4). If these phenomena also occur in feedlot cattle then the potential exists for

reducing the prevalence of *E. coli* O157 in feedlot cattle through management modulation.

The purposes of the present study were to estimate the prevalence of *E. coli* O157 in feedlot cattle in the USA, to estimate the fraction of feedlots in which the agent exists (feedlot prevalence), and to determine the distribution of *E. coli* O157 in United States feedlot cattle with respect to geographic region and stage of feeding.

MATERIALS AND METHODS

The present study was performed in the states of Arizona, California, Colorado, Idaho, Illinois, Iowa, Kansas, Minnesota, Nebraska, Oklahoma, South Dakota, Texas, and Washington as part of the USDA, APHIS, VS National Animal Health Monitoring System's Cattle on Feed Evaluation (COFE) project. A stratified random sample from a list from the National Agricultural Statistics Service was used to identify candidate feedlots. A total of 1,411 feedlots participated in the core COFE project, of which 498 had a >1,000-head one-time capacity. *E. coli* sampling was carried out in 100 of these 498 larger feedlots; 116 feedlot owners were contacted to reach the number of 100 (16 declined to participate). Each feedlot was sampled on one occasion between 1 October 1994 and 20 December 1994. In each selected feedlot, 30 fresh fecal pats were sampled from each of the following: pens with cattle on feed for the shortest time, pens with cattle the longest time on feed, and each of two randomly selected pens.

Fresh fecal pats (less than approximately 2 h after defecation) were sampled by evenly coating a cotton-tipped swab with fecal material and placing it into a screw-cap tube containing a transport medium; the tubes with samples were maintained on ice packs in insulated shipment containers. All samples were shipped by overnight delivery to the assigned laboratory on the day of collection. Two laboratories were utilized in the study (arbitrarily designated laboratories 1 and 2): Washington State University's Field Disease Investigation Unit laboratory at Pullman, Washington, USA, and the National Veterinary Services Laboratories at Ames, Iowa, USA. Samples from 40 and 60 of the selected feedlots were tested at laboratories 1 and 2, respectively. The 40:60 ratio of feedlots from which samples were analyzed at laboratories 1 and 2 was maintained in each geographical region, and the sampling in each region for each lab was spread roughly evenly throughout the sampling period. Two laboratories were used due to the large

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number of samples (11,881) to be assayed over such a short period of time (3 months).

Sample swabs from feedlots tested by laboratory 1 were collected and shipped in 3 ml of tryptic soy broth (Difco Laboratories, Detroit, MI) with the addition of 50 ng of cefixime per ml (Wyeth-Ayerst Research, Pearl River, NY) and 40 µg of vancomycin per ml (Abbott Laboratories, Chicago, IL) (TSB_{CV}). Within 24 h of arrival at the laboratory fecal samples were briefly agitated and then incubated for 18 to 24 h at 37°C. Dilutions to 10⁻⁴ were prepared in TSB and 300 µl of both a 10⁻³ and 10⁻⁴ dilution were each plated onto separate 150 mm sorbitol MacConkey agar plates (Difco) containing 50 ng of cefixime per ml and 2.5 µg of potassium tellurite per ml (Sigma Chemical Co., St. Louis, MO) (SMAC_{CT}) using sterile glass spreaders. SMAC_{CT} plates were incubated overnight at 37°C, and up to 10 non-sorbitol-fermenting colonies per sample were transferred to MacConkey agar plates (Difco) to evaluate lactose fermentation. Colonies that were sorbitol negative and lactose positive were tested for beta-glucuronidase activity using a 4-methyl-umbelliferyl-beta-D-glucuronide (MUG) (Sigma) assay. MUG-negative colonies were then transferred to SMAC agar plates and assayed for O157 antigen using a latex agglutination assay (Unipath Limited, Basingstoke, Hampshire, UK). All *E. coli* O157 isolates were assayed for Shiga-like toxin-coding genes by using DNA-DNA hybridization (9, 13) and only those that possessed these genes were included as *E. coli* O157 in this study.

The culture methods used by laboratory 2 were similar to those in laboratory 1 except that samples were collected in 1 ml of Cary-Blair transport medium (prepared in house) and stored at 4°C for up to 10 (mean, 3.6) days prior to culturing. For bacteriological culture 2 ml of modified EC broth (Difco) containing 20 µg of novobiocin per ml (Sigma) was added to each sample immediately prior to an overnight incubation at 37°C. Flagellar antigen (H) typing was done at laboratory 2 for all *E. coli* O157 isolates using H-specific antisera (prepared in house). All *E. coli* O157 isolates were assayed for Shiga-like toxin-coding genes using the DNA-DNA colony hybridization described elsewhere (7).

The Kruskal-Wallis nonparametric test was used to assess statistical differences in prevalence between labs and among geographic regions (5). For these analyses, the unit of observation was considered to be the feedlot. Statistical significance of differences among days-on-feed groups were tested using the Kruskal-Wallis test with pen as the unit of observation. For testing region and days-on-feed effects, data from the two laboratories were pooled. The significance of clustering of *E. coli* O157 was tested using deviation from the expected binomial distribution (5).

RESULTS

Of the 11,881 fecal samples tested at the two laboratories, 188 (1.58%) were found to be positive for *E. coli* O157:H7 with an additional 22 (0.2%) being positive for *E. coli* O157:H- (Table 1). *E. coli* O157 was detected in 113 of 4768 (2.4%) fecal samples at laboratory 1 and in 97 of 7113 (1.4%) at laboratory 2 (Table 1); the difference in measured prevalence between the two laboratories was significant ($P < 0.01$). *E. coli* O157 was isolated from 63 of 100 (63%) of feedlots; *E. coli* O157:H7 was isolated from 61 of 100 feedlots (61%).

No evident regional distribution of *E. coli* O157 was observed (Table 1). Samples submitted to laboratory 1 suggested a somewhat (but not significantly; $P = 0.14$) higher prevalence among southern-region feedlots, but this

trend was not observed among feedlot samples tested at laboratory 2.

The prevalence of *E. coli* O157 within feedlots ranged from 0% to 10% (Fig. 1). The distribution of within-feedlot prevalence significantly ($P < 0.01$) deviated from chance

TABLE 1. Prevalence of *E. coli* O157 in cattle feces in 100 feedlots by region and laboratory

Region ^a	Prevalence in fecal samples: no. positive/tested (%) tested in:			
	Laboratory 1		Laboratory 2	
	<i>E. coli</i> O157 ^b	<i>E. coli</i> O157:H7	<i>E. coli</i> O157	<i>E. coli</i> O157:H7
Southern (Arizona, ^{cd} California, ^d Texas ^{cd})				
Individual fecal samples	46/1440 (3.2)	45/1440 (3.1)	37/2344 (1.6)	28/2344 (1.2)
Feedlots	11/12 (92)	11/12 (92)	11/20 (55)	11/20 (55)
Median within-feedlot prevalence (Q1, Q3) ^e	(2.5) (1.7,2.9)	(2.5) (1.7,2.9)	(1.3) (0.0,1.7)	(0.8) (0.0,1.7)
Middle (Colorado, ^c Kansas, ^{cd} Okla- homa ^d)				
Individual fecal samples	23/1558 (1.5)	18/1558 (1.2)	39/2280 (1.7)	34/2280 (1.5)
Feedlots	9/13 (69)	8/13 (62)	10/19 (53)	9/19 (47)
Median within-feedlot prevalence (Q1, Q3)	(0.8) (0.0,1.7)	(0.8) (0.0,1.7)	(0.8) (0.0,2.5)	(0.0) (0.0,2.5)
Northern (Idaho, ^d Illinois, ^d Iowa, ^d Minnesota, ^c Nebraska, ^{cd} South Dakota, ^d Washington ^{cd})				
Individual fecal samples	44/1770 (2.5)	42/1770 (2.4)	21/2489 (0.8)	21/2489 (0.8)
Feedlots	12/15 (80)	12/15 (80)	10/21 (48)	10/21 (48)
Median within-feedlot prevalence (Q1, Q3)	(1.7) (0.8,4.4)	(1.7) (0.8,4.4)	(0.0) (0.0,0.8)	(0.0) (0.0,0.8)
Total				
Individual fecal samples	113/4768 (2.4)	105/4768 (2.2)	97/7113 (1.4)	83/7113 (1.2)
Feedlots	32/40 (80)	31/40 (78)	31/60 (48)	30/60 (50)
Median within-feedlot prevalence (Q1, Q3)	(1.7) (0.8,2.9)	(1.7) (0.8,2.9)	(0.8) (0.0,1.7)	(0.4) (0.0,1.7)

^a Regional differences not significant ($P > 0.10$).

^b Included O157:H-.

^c Feedlots from this state assayed at laboratory 1.

^d Feedlots from this state assayed at laboratory 2.

^e Quartile 1 and quartile 3 prevalences.

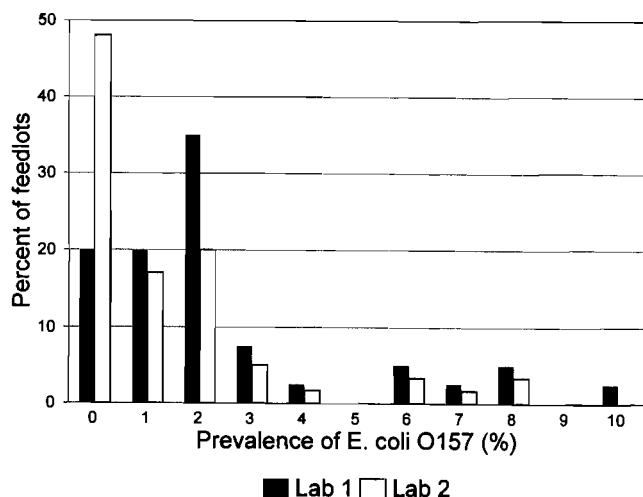


FIGURE 1. Prevalence of *E. coli* O157 found in fecal samples among 100 feedlots tested at two different laboratories.

TABLE 2. Relationship between *E. coli* O157 presence and stage on feed of cattle by pen and by individual fecal-pat samples for Laboratories 1 and 2

Stage on feed ^a of cattle in pens	<i>E. coli</i> O157 presence: (no. positive/tested (%)) in:			
	Lab 1		Lab 2	
	Pens	Samples	Pens	Samples
Shortest on feed	21/40 (52.5)	55/1200 (4.6)	19/60 (31.7)	46/1788 (2.6)
Randomly selected	21/79 (26.6)	42/2370 (1.8)	17/120 (14.2)	34/3555 (1.0)
Longest on feed	8/40 (20)	16/1198 (1.3)	8/60 (13.3)	17/1770 (1.0)

^a Shortest-on-feed pens had significantly higher median prevalence than randomly selected or longest-on-feed pens ($P < 0.01$).

aggregation, indicating temporal, spatial, and/or exposure (i.e., risk factor) clustering.

E. coli O157 prevalence was markedly higher in the shortest-on-feed pens compared to either randomly selected ($P < .01$) or longest-on-feed pens ($P < 0.01$) (Table 2). This effect was consistent for feedlots tested at both laboratories 1 and 2, in spite of the overall higher prevalence of *E. coli* O157 detected by Lab 1. The average values and standard deviations for days-on-feed for shortest- and longest-on-feed pens were 7.0 ± 11.1 and 185.0 ± 69.4 , respectively.

DISCUSSION

The prevalence of *E. coli* O157 observed in feedlot cattle in the present study is substantially higher than that of a previous study (3) which used culture methods that were less sensitive than several more recent studies (4, 12). Furthermore, only 4 feedlots were included in the earlier study (3).

The prevalence of *E. coli* O157 observed in the present study was also substantially higher than those in several reports in dairy and range cattle (1, 3), although it is similar to that reported in another study which involved dairy cattle

(4). It seems probable that much of the diversity in the reported prevalence of *E. coli* O157 among different cattle types is associated with differences in the number of samples evaluated per herd, the detection methods used (14), and the age differences of the animals sampled. In a dairy study in which 360 fecal samples per herd were collected over a period of 6 months, 78% of herds were found to have one or more positive samples (4). Together with the results of the present study these data are most consistent with a ubiquitous distribution of *E. coli* O157 in cattle operations.

It is also evident from the data of the present study that *E. coli* O157 is broadly distributed across the United States, a conclusion which is in agreement with previous data (1). These findings contrast with reports of human disease due to *E. coli* O157, which have been more common in the northern United States (2).

The evidence of significant clustering at the pen and feedlot level could be the result of temporal clustering within feedlots such that a relatively high prevalence of *E. coli* O157 occurs during intermittent bursts of *E. coli* O157 fecal shedding which are separated by relatively longer periods of low (or zero) prevalence. This could account for the skewed distribution of Figure 1. It is also possible that the clustering was the result of exposure factors which differed among the feedlots. In this scenario, those feedlots with certain levels of critical-exposure factors (for example, a feed-ingredient variable) would have higher levels of *E. coli* O157 excretion in cattle. An important weakness of a prevalence study with only one sampling visit per feedlot, as in the present study, is that it is impossible to distinguish between temporal and exposure clustering.

The finding of an approximately threefold higher prevalence of cattle excreting *E. coli* O157 during the early feeding period is potentially of major significance, but its cause is uncertain. It is possible that the higher prevalence in the shortest-on-feed pens is the result of dietary stress associated with adaptation to feedlot rations. Rasmussen et al. (12) demonstrated that dietary stress can result in replication of *E. coli* O157 in rumen fluid. Alternatively the effect could be the result of gastrointestinal floral disturbances associated with morbidity and antibiotic treatment of cattle, both of which are relatively concentrated in newly arrived cattle.

In the present study the flagellar H antigen designation was not considered important in differentiating *E. coli* O157. This decision was based on research indicating that H7 is not a consistent clone marker (18). Failure to ferment sorbitol, a lack of beta-glucuronidase, possession of Shiga-like toxin-coding genes, and the possession of O157 antigen have been reported to be reliable markers for what has been designated the DEC-4 clone of *E. coli* (18). All isolates reported as *E. coli* O157 in the present study possessed these markers.

Although the results suggested a sensitivity difference between the two laboratories, the study was arguably enhanced by having independent samples assayed at two separate laboratories. The failure to observe a significant regional effect in *E. coli* O157 prevalence among feedlots tested by either laboratory adds to the weight of evidence against a pronounced geographical difference in *E. coli*

O157 prevalence. Similarly the threefold higher prevalence of *E. coli* O157 excretion in newly arrived cattle was supported by results from both laboratories.

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